



AGENCE FRANÇAISE  
DE SÉCURITÉ SANITAIRE  
DES ALIMENTS

*Site de  
Maisons-Alfort*

LABORATOIRE D'ÉTUDES  
ET DE RECHERCHES SUR  
LA QUALITÉ ALIMENTAIRE  
ET SUR LES PROCÉDÉS  
AGROALIMENTAIRES



***EU COMMUNITY REFERENCE  
LABORATORY FOR  
MILK & MILK PRODUCTS***

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# 2008 Technical report of the Community Reference Laboratory for Milk and Milk Products

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## Introduction

The AFSSA-LERQAP (Laboratory for Studies & Research on Quality of Foods & on Food Processes) has undertaken, as Community Reference Laboratory (CRL) for milk, the following works in 2008 according in particular to (a) the actions planned at the 10<sup>th</sup> Workshop of the National Reference Laboratories (NRLs) (28&29 June 2007), and (b) the work programme defined in Annex I of the Framework Partnership Agreement between EC/DG SANCO and the CRL for the period 2006-2010.

These actions are part of the new role of the CRL, restricted to the control of raw and heat-treated liquid milk (total flora, somatic cells count, phosphatase activity), as well as cheeses for phosphatase, in the frame of the Regulation 853/2004 *laying down specific hygiene rules for food of animal origin*.

The Annex III, Section IX of Regulation 853/2004 is dedicated to raw milk and dairy products:

- Microbiological criteria on total flora at 30°C and on somatic cells count are fixed for:
  - o raw cow's milk and for raw milk from other species milk (Chapter I, III),
  - o dairy products (Chapter II, III-criteria for the use of raw cow's milk for further processing).
- Phosphatase activity:
  - o In Chapter I, I.3, a reference is made to a negative phosphatase test to characterize the heat-treatment applied to certain raw milks at the primary production stage (Chapter I, I.3).
  - o Chapter II(II) includes requirements on heat-treatment of raw milk or dairy products, applicable to food business operators. A cross reference is made to the general requirements of Regulation 852/2004, Annex II, Chapter XI.

The CRL has in particular provided a support to the NRLs for the implementation of:

- the Regulation 853/2004;
- the derived Regulation 1664/2006, recently published, defining amongst other the testing methods for raw milk and heat-treated milk to be used by competent authorities and food business operators:
  - o to check compliance with the limits for total flora and somatic cells count laid down in Regulation 853/2004, Annex III/Section IX/Chapter I/Part III,
  - o to ensure appropriate application of a pasteurisation process to dairy products, as referred to in Regulation 853/2004, Annex III/Section IX/Chapter II/Part II.

*NB: In brackets under each item, the scheduled duration of the action is indicated: either annual (limited to 2008) , either multi-annual (on-going programme on several years).*

# 1. Inter-laboratory proficiency testing

## 1.1 Somatic cells count (annual)

**Frame** : In Section IX of Regulation 853/2004, microbiological criteria have been fixed for raw milk (Chapter I, III) and dairy products (Chapter II, III-criteria for the use of raw cow's milk for further processing). They include criteria on somatic cells count for raw cow's milk and from other species.

The Regulation 1664/2006 prescribes the reference method for somatic cells count, Standard ISO 13366-1 as well as conditions for the use of alternative methods.

The CRL (Unit HMPA) has organised in 2008 an inter-laboratory trial on somatic cells count by the reference method, the Standard EN ISO 13366-1 (microscopic method). 25 NRLs took part to the trial.

The CRL (Unit HMPA) has prepared and dispatched in advance (Week 48) the instructions and test report for the trials. CECALAIT has prepared the samples (7 samples of raw milk with a different given level of somatic cells, and 1 microscopic slide) and dispatched them in December (Week 49) to the participants.

The CRL has collected the results and is currently preparing the report to be soon dispatched.

## 1.2 Determination of alkaline phosphatase activity (annual)

**Frame:** The Regulation 1664/2006 defines the reference method, the Standard ISO 11816-1, the legal limit for negativity of the test (350 mU/l for cow's milk) and conditions to use alternative methods.

### *a. Criteria to evaluate the individual performance of NRLs*

The working group on statistics applied to the performance evaluation in PT trials met in Brussels on 24 January and decided:

- that the assigned value would be the robust mean,
- that the standard deviation for the proficiency assessment ( $\sigma_p$ ) would be the standard deviation obtained from the participants results,
- to calculate z-scores for each replicate and for all results, even those not respecting the repeatability limit.

### *b. PT trial*

CRL has drafted the report on the PT trial organized in November 2007 according to the criteria defined above. The trial prescribed use of the fluorometric reference method on cow's whole, semi-skim and skim milk.

## 2 Analytical development

These works are conducted in the CRL laboratory alone.

### 2.1 Determination of total flora at 30°C in raw milk (multi-annual)

**Frame** : The Regulation 1664/2006 prescribes the reference method for total flora at 30°C, Standard EN ISO 4833 as well as conditions for the use of alternative methods.

#### *a. Coordination of the NRLs*

The CRL has circulated a new questionnaire to the NRLs, by circular letter of 14/01/2009, on national practices on the use of alternative methods for determination of total flora in raw milk, and on the conversion factors in particular. The outcome of this questionnaire will enable to prepare the 2010 workshop to be devoted to alternative methods for raw milk hygiene.

The CRL has prepared a draft check-list for the NRLs to conduct the visits of laboratories in charge of establishing the conversion factors. It has submitted it to the D and BE NRLs, got comments and will circulate it to the NRLs, after getting the opinion from the IDF/ISO project leader for the Standard ISO 21187.

#### *b. Study of the alternative methods*

The CRL (Unit HMPA) had launched experimental studies in 2007 using a flow cytometer (Bactocount) as an alternative method to the bacterial count, in order to investigate the questions linked to the correlation of this method to the reference method, especially the different factors influencing, for a same apparatus, the value of the conversion factor (period of lactation, type of feeding,...).

The study progressed in 2008. Batches of raw milk were delivered at regular intervals of time and analysed in parallel by the reference method EN ISO 4833 and by the Bactocount. In total 330 samples were analysed in 2007 and 450 samples was analysed in 2008.

It is intended to finish the study in 2009.

#### *c. Scientific and technical support*

The CRL coordinator has followed the IDF/ISO the revision of the Standard IDF 161 detailing the specificities of validation of a routine method against a reference method for the determination of total flora in raw milk.. The works are still at an initial stage and no significant progress has been done in 2008.

## **2.2 Determination of total flora at 30°C in colostrums** (multi-annual)

**Frame:** DG SANCO intends to add hygienic requirements for colostrums in Regulation 853/2004. But the CRL and NRLs consider that data on levels of total flora in colostrums are not readily available.

In 2008, the CRL has prepared the experimental design for the new study on total flora in colostrums and has investigated the way to get appropriate samples from farms.

## **2.3 Determination of somatic cell count in raw milk** (multi-annual)

**Frame :** The Regulation 1664/2006 prescribes the reference method for somatic cell count in raw milk, Standard ISO 13366-1 as well as conditions for the use of alternative methods.

The CRL (Nicole Soudrie and Alexandra Cauquil, Unit HMPA) followed a training session dedicated to the reference method for somatic cells count in raw milk, Standard ISO 13366-1.

The CRL has analysed with the Bactocount raw milk samples delivered regularly and analysed also for total flora (see 2.1), as well as analysed once per month guaranteed-content samples from CECALAIT.

## **2.4 Determination of alkaline phosphatase activity** (multi-annual)

The CRL staffs working on AP have received *in situ* a training course by the company manufacturing the Fluorophos instruments (Advance Instruments). The aim was to enable the team to ensure themselves part of the maintenance and revision of the Fluorophos instruments in the laboratory. This was also the occasion to discuss scientific aspects related to the method and to envisage revision of the ISO/IDF International standard (EU reference method) in order to improve the performances of the method.

### *a. Determination of the phosphatase activity in milk other than cow's milk*

The CRL (Unit CALAS) has continued the study of AP levels for species other than cow. The purpose of this work is to support DG SANCO in prescribing legal limits of AP activity in pasteurised milk from different species.

#### Goat's milk

The CRL has conducted a large number of analyses on raw milk and on the same milk after laboratory pasteurisation at 63°C for 30 minutes.

The CRL has analysed 12 samples from Cyprus (analysed in parallel by the Cyprus NRL), 4 samples from Slovakia, 20 from Romania and 7 samples from Greece.

In view of the results concerning the Romanian and Greek samples, the CRL has asked the relevant NRLs to send complementary series of samples (6 samples from Romania, 12 samples from Greece) for AP determination in raw and laboratory-pasteurised samples with a

simultaneous evaluation of the microbiological quality of the pasteurised samples. Microbiological analyses were carried over by the Unit HMPA and dealt with the determination of total flora and *Enterobacteriaceae*.

### Ewe's milk

As acknowledged at the targeted AP NRLs Workshop (Vienna, 9&10 October), the unexpectedly heavy work load resulting from the studies on goat's milk lead to the impossibility to tackle the issue on ewe's milk in 2008.

### Camel's milk

A preliminary mission was achieved in Central Veterinary Research Laboratory (CVRL) of Dubai in March 2008 in order to establish face-to-face contact, visit the laboratory and share technicalities with the technical staff, visit the camel farm and the factory producing pasteurized camel milk, and especially have a scientific exchange and discussion on the collaboration project between CRL and CVRL.

Raw camel's milk samples and laboratory-pasteurized samples were shipped to CRL under cold conditions. A very satisfactory equivalence of results of phosphatase activity was obtained for the samples pasteurized in Dubai and those heat-treated in CRL. The data obtained seem to prove that phosphatase is not a pertinent marker for correct pasteurization of camel milk.

#### *b. Determination of alkaline phosphatase activity in cheese*

The CRL (Marina NICOLAS) introduced during the meeting of the ISO/IDF Joint Action Team of experts (Analytical ISO/IDF Week, Switzerland, May 2008) a paper explaining the need to revise the international ISO/IDF Standard and expanded on the critical points that ought to be improved. The proposal was accepted by the experts, the CRL will draft a first version of the revised standard that will be discussed during Analytical Week 2009.

The CRL continued and finalised the study on residual AP activity in soft cheese from pasteurized milk. Some samples of pasteurized hard cheese were also analyzed. The evaluation of the data obtained lead to the proposal of a tentative limit for AP in pasteurized cheese of less than 10 mU/g. This limit was submitted to NRLs during the AP NRLs Workshop.

At the workshop, the Italian NRL presented valuable data on Italian cheese that proved that the tentative limit was applicable for Italy. Same applies to the Swiss NRL.

All other NRLs were requested to control their national cheese production in order to confirm, or not confirm, acceptability of the proposed regulatory limit.

#### *c. Comparison of the chemiluminescent and the fluorimetric methods*

The CRL conducted in 2008 a new comparison study between the chemiluminescent method (Novalum) and the current regulatory reference method, the fluorimetric method (Fluorophos). More than fifty samples of cow's and goat's milk have been tested with both protocols. This study showed an improvement but there is still a non-negligible bias between

the two methods. Presently, it is not yet possible to conclude to an equivalence between the official and the alternative methods.

The developer of the alternative method (Charm Science, USA) visited the CRL in May 2008 and expressed satisfaction regarding the way his method is operated by CRL and the robustness of the studies conducted.

*d.      Reactivation of AP*

A very complete bibliographic survey on reactivation of AP was conducted by the CRL in 2008, in preparation of the AP NRLs Workshop.

### **3 Assistance to the NRLs**

#### **3.1 Training courses**

There has been no individual request from NRLs for training in 2008.

### **4 NRLs Workshop**

The CRL organised the 11<sup>th</sup> NRLs Workshop dedicated on alkaline phosphatase activity, on 09 & 10h October 2008 in Vienna (Austria). It enabled:

- to make an update of all the works undertaken by the CRL on this topic, especially since the former workshop dedicated to this topic (2002);
- to envisage the works to be conducted for the following years.

### **5 Technical and scientific assistance to the European Commission**

#### **5.1 Participation to ISO/IDF standardization works**

On behalf of DG SANCO, participation of Marina NICOLAS and the CRL Coordinator (Bertrand LOMBARD) to the IDF/ISO works on the analytical methods specific to the analysis of raw milk, and in particular:

- microbiological analysis of milk & dairy products;
- somatic cells count: reference and alternative methods,
- total flora: alternative methods,
- phosphatase test: reference and alternative methods.

Participation of M. Nicolas and B. Lombard to the IDF/ISO Analytical Week (Montreux, 19-23 May) and the meeting of the groups dealing with the topics mentioned above. In particular M. Nicolas chaired the meeting of the ISO/IDF Joint Action Team of experts on Heat Treatment and also chaired the meeting of the Standing Committee on Minor Components and physical properties.

## **6 Publications and CRL reports**

Cauquil A., Soudrie N., Maladen V., Deperrois V., Asséré A., Lombard B. (2008) Inter-laboratory proficiency testing study on the enumeration of microorganisms in milk by colony-count technique at 30°C. Community Reference Laboratory for milk

Report on the inter-laboratories Proficiency Testing on Alkaline Phosphatase activity in cow's milk organised on November 2007 (30/09/08), Anne-Cécile Boitelle and Marina Nicolas.